Final Proposal

Enhancing PCA-SIM for Robust Dynamic Structured Illumination Tracking in Long-Term Live-Cell Imaging

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Outline

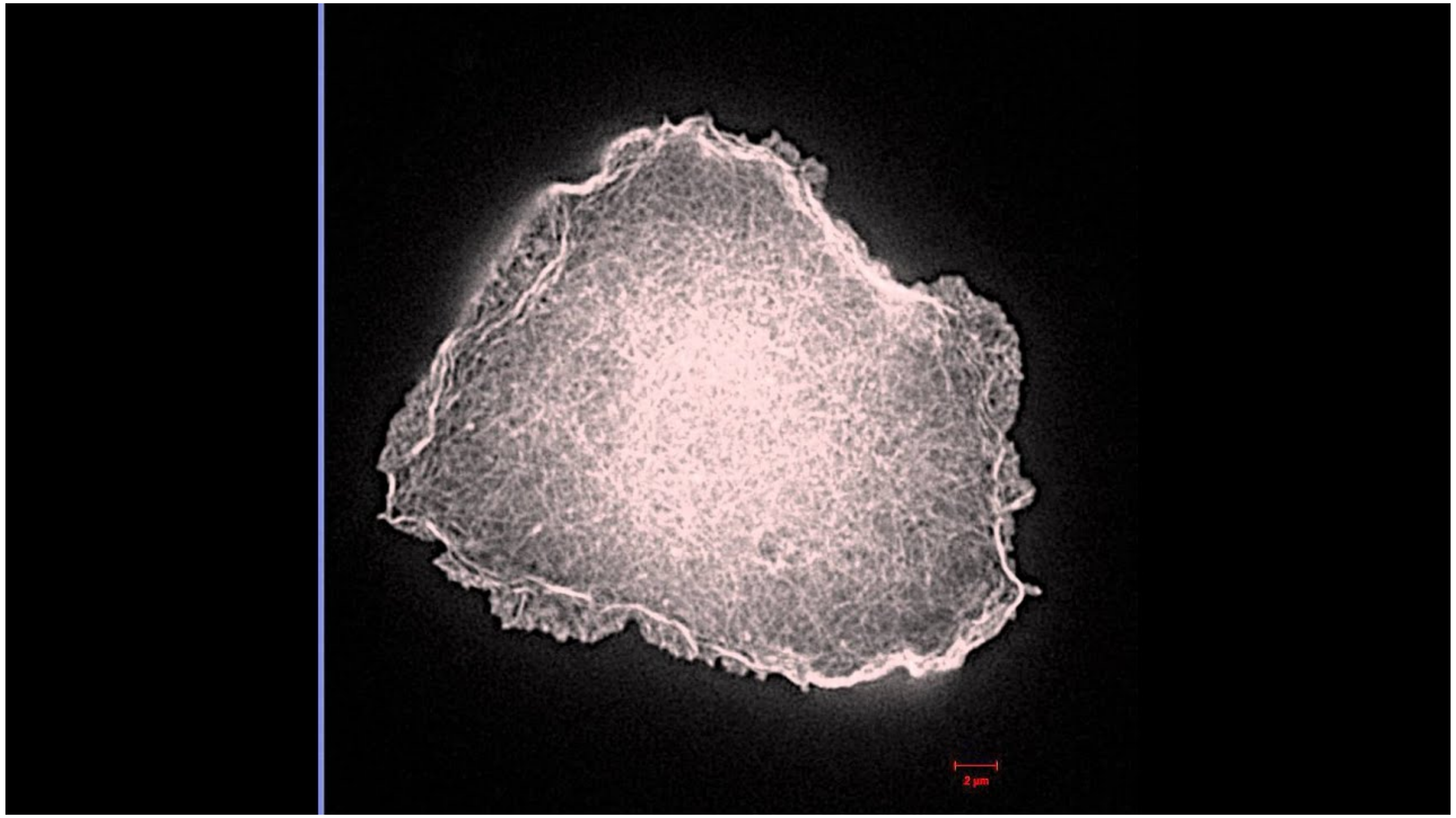
SIM on Live-cell Imaging

SIM (Structured Illumination Microscopy)

- Minimal Phototoxicity
- Minimal Photobleaching

=> **Live-cell Imaging**

- High Speed (e.g. JSFR-AR SIM)
- Large FOV
- Small data size



Live cell SR-SIM imaging of SR2+ cell labelled with Lifeact-EGFP (4.5 sec for each time point).The authors visualized the actin-driven lamellipodial membrane dynamics inside live cells, they were able to resolve changes of actin structures in a multicellular context up to 70 µm inside a Drosophila egg chamber.

Reference: https://www.youtube.com/watch?v=-LuYXE8Wq7I

Problem

SIM fails over time?

- Traditional SIM requires precise knowledge of illumination patterns (frequency, phase, orientation…)
- PCA-SIM (2023) can estimate these parameters with remarkable precision:

  => Frequency errors less than 0.01 pixels and phase errors under 0.1% of 2π.

- However, this precision degrades over time… (Long-term imaging may take hours or even more.)

  A. Mechanical drift

  B. Thermal expansion

  C. Optical components

  D. Sample-induced aberrations (Live-cell), optical path shift…

Overview on PCA-SIM

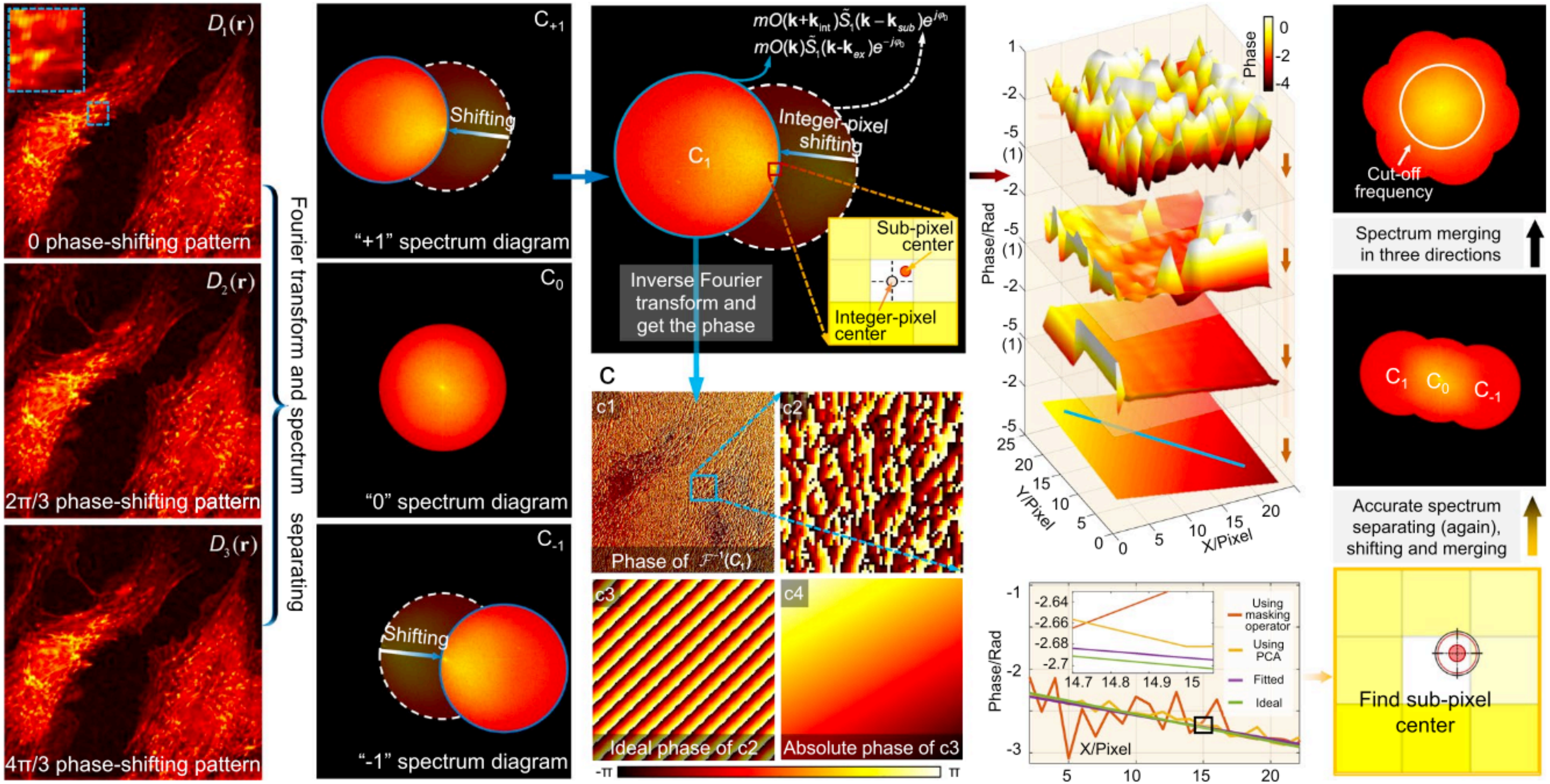
Structured illumination microscopy based on principal component analysis
J Qian, Y Cao, Y Bi, et al.
Published February 07, 2023

=> A SIM reconstruction algorithm that uses principal component analysis to estimate illumination pattern parameters (frequency vector, phase, etc.)

Dynamically estimating illumination parameters for each frame, rather than relying on fixed calibration.

Observation: Ideal phasor matrix of a SIM pattern is of rank one.
=> Rank-1 matrix can be expressed as the outer product of two vectors.
=> Indicates respectively the x and y components.
=> Should have only one principal component.
(Single best subspace of the data)

Overview on PCA-SIM



Overview on PCA-SIM

1: Obtain the Fourier spectrums of the raw SIM images

2: Separate the 0- and ±1-order spectrums and shift the ±1-order spectrums with integer-pixel displacement

3: Use a masking operator to extract the center signals for inverse Fourier transform and obtain the exponential term

4: SVD and extract the principal component

5: Fit two principal vectors with the least square method after removing starting error points

6: Obtain accurate sub-pixel wave vector

7: Obtain the initial phase and modulation depth

8: Merge separated spectrums and perform super-resolution reconstruction

PCA-SIM - Strength & Limitation

- Strength:

  1. A non-iterative approach (fast) with precise parameter estimation (frequency errors less than 0.01 pixels and phase errors under 0.1% of 2π.).

  2. A dynamic estimation.

  3. Can still estimate parameters under low SNR with real-time imaging.

- Limitation (especially for long-term live-cell imaging):

  1. Each frame is processed independently, ignoring temporal continuity.

  2. Noisy estimation under low SNR scenarios.

  3. Rank-1 phasor assumption might struggle with complex aberrations.

Proposed Solution

Component 1: Streamed/Windowed PCA for temporal adaptation:

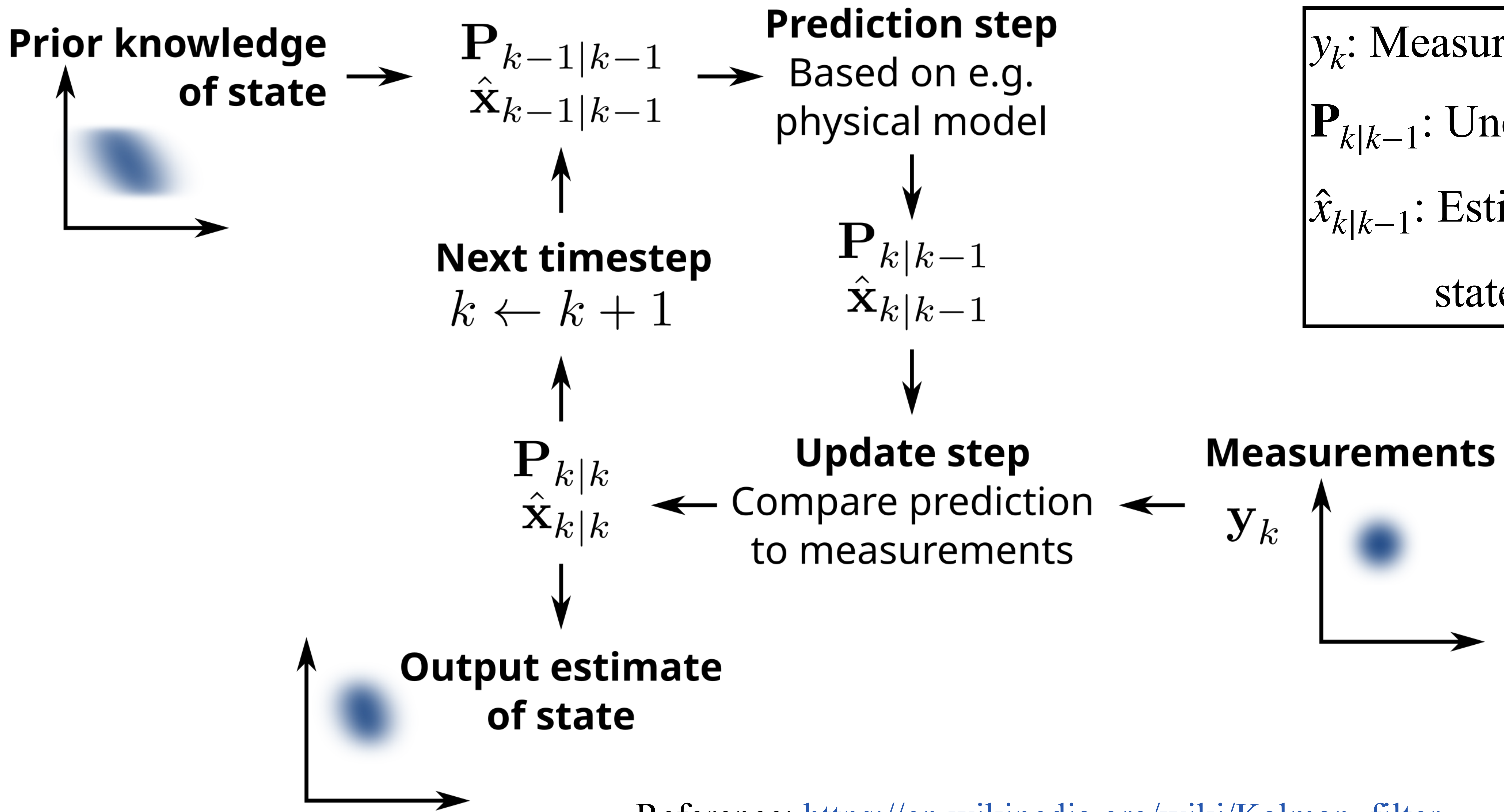
- At each new time point, combine the newest raw SIM images with a few preceding frames' data to form the phasor matrix for PCA.
  1. Averaging the phasor matrices of the last N frames.
  2. Concatenating them in a larger PCA analysis.
- Exponential forgetting factor

Hierarchical pyramid

1. Random noise can be reduced (averaging).
2. Tunable window length provides a trade-off between responsiveness and stability.
3. Slow drifts in frequency (phase) manifest as slight shifts in the principal component over the window.

Proposed Solution

Component 2: Kalman Filtering



y_k: Measurements

$\mathbf{P}_{k|k-1}$: Uncertainty

$\hat{x}_{k|k-1}$: Estimation of the system's state at time k before y_k

Reference: https://en.wikipedia.org/wiki/Kalman_filter

Proposed Solution

Component 2: Kalman Filtering

Recursive Estimation:

- Prediction Step: Using control commands to predict where the system will be in the next time point.
- Correction Step: Utilizing sensor observations to correct for potential mistakes

Assumptions:

- Gaussian world. (Belief, uncertainty, …, are all gaussians.)
- All models are linear.

→ Optimal Estimator

Reference: https://www.youtube.com/watch?v=o_HW6GnLqvg

Proposed Solution

Component 3: Closed-Loop Hardware Feedback

Modern SIM setups project structured patterns by:

1. Spatial Light Modulators (SLMs)
2. Digital micromirror devices (DMDs)
3. Galvanometer-scanned diffraction gratings

   => computer-controlled and can be adjusted on the fly.

- Use the computed illumination parameters from PCA-SIM (after Kalman filtering) as feedback to tweak the hardware and correct any deviations in the pattern in real-time.

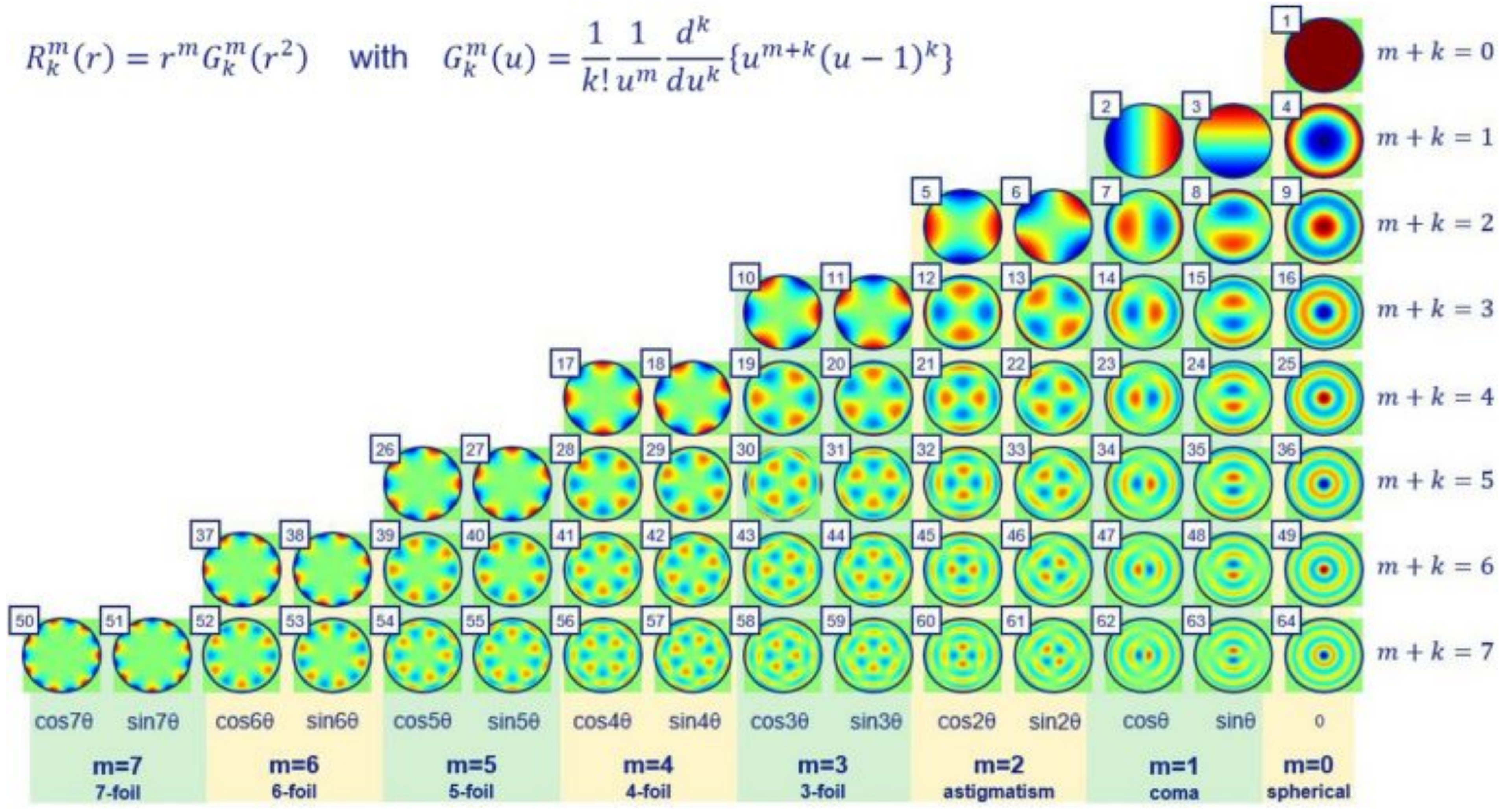
Proposed Solution

Component 4: Physics-Informed Deep Learning

- To handle cases that are hard to model explicitly (by largely model-based and analytical steps)

- Initializer and outlier detector for the parameter estimation [2] with RNN (Transformer).
  => A supplement to the Kalman filter, improving prediction in non-linear drift scenarios.
- Aberrations corrector using CNN with Zernike aberration coefficients as outputs.
  => Input: Filtered SIM images ; Output: refined estimate of the illumination phasor.

* Or leverage Hierarchical Reinforcement Learning for more interpretable implementation. [4]

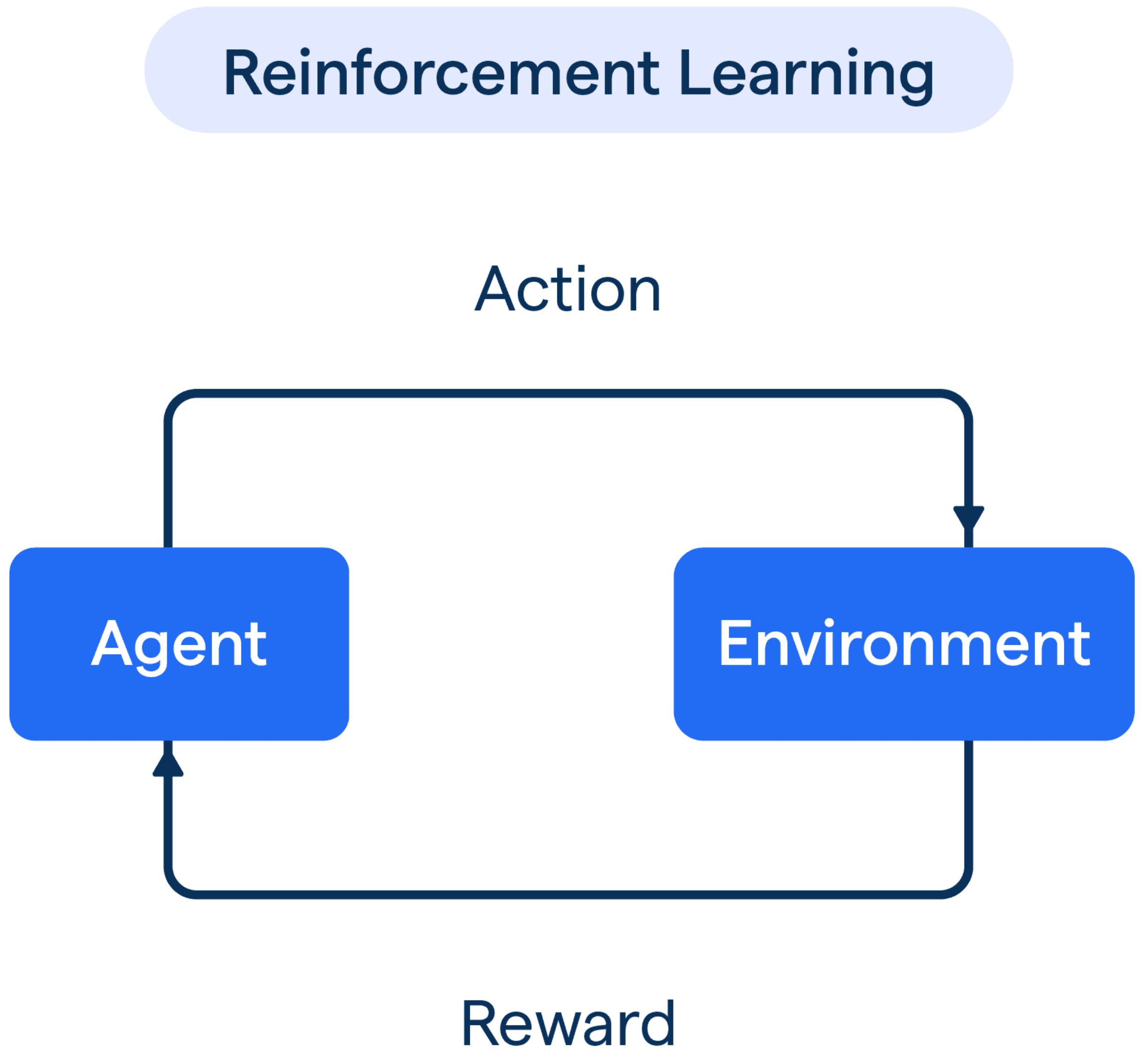


Reference: https://radojuva.com/en/2024/08/spherical_aberration_and_lens_bokeh/

Proposed Solution

Component 4: Physics-Informed Deep Learning

- Hierarchical Reinforcement Learning [4]



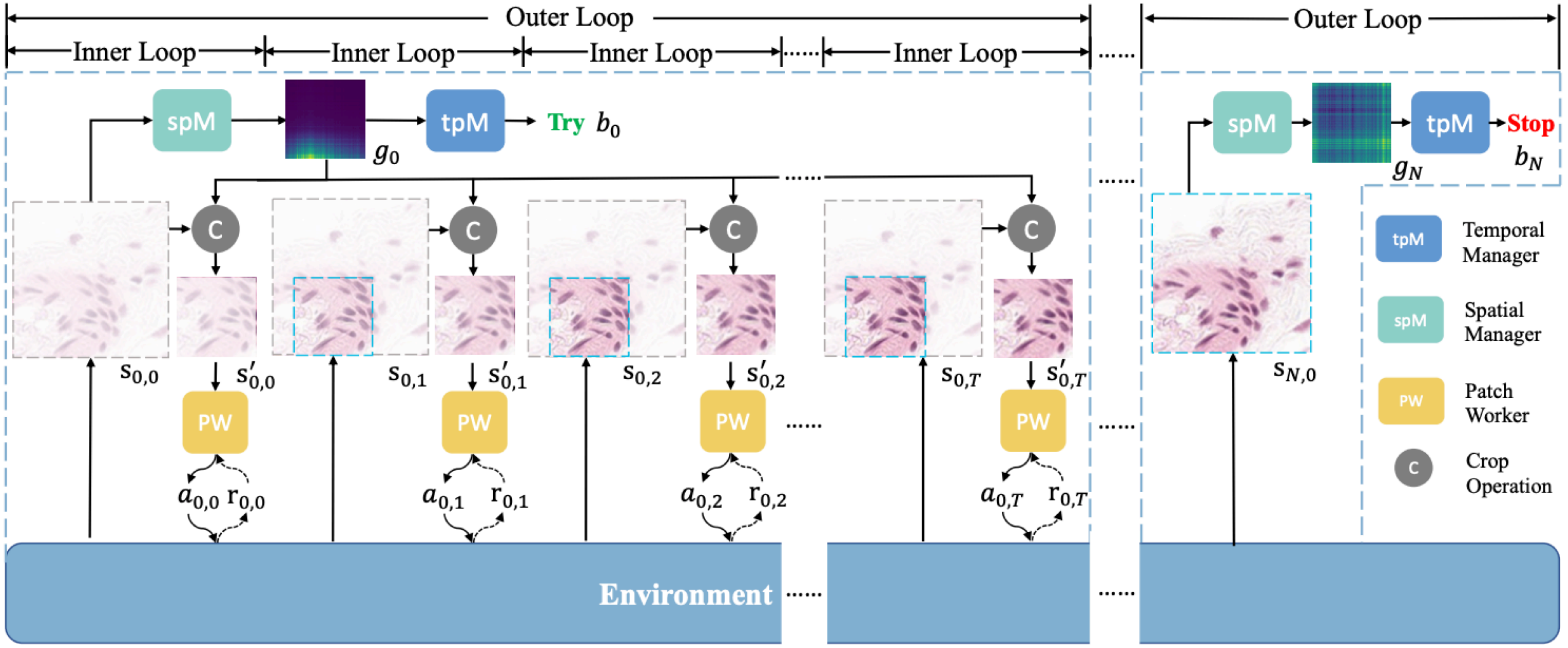


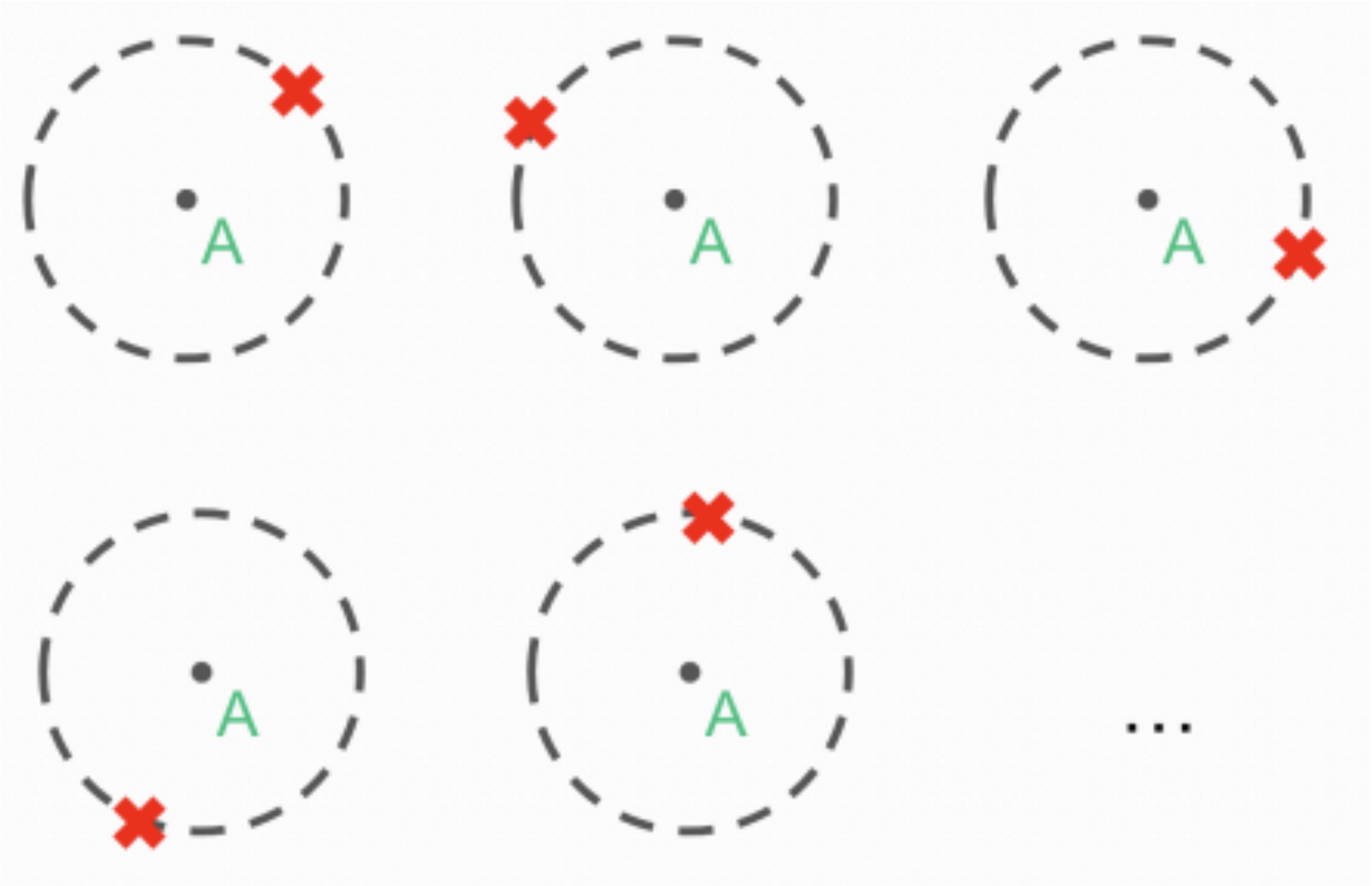
Fig. 3: The overview of unrolled Spatial-Temporal hierARchical Reinforcement Learning (STAR-RL) framework, consisting of an outer loop for patch selection and several inner loops for patch recovering.

Reference: https://botpenguin.com/glossary/reinforcement-learning

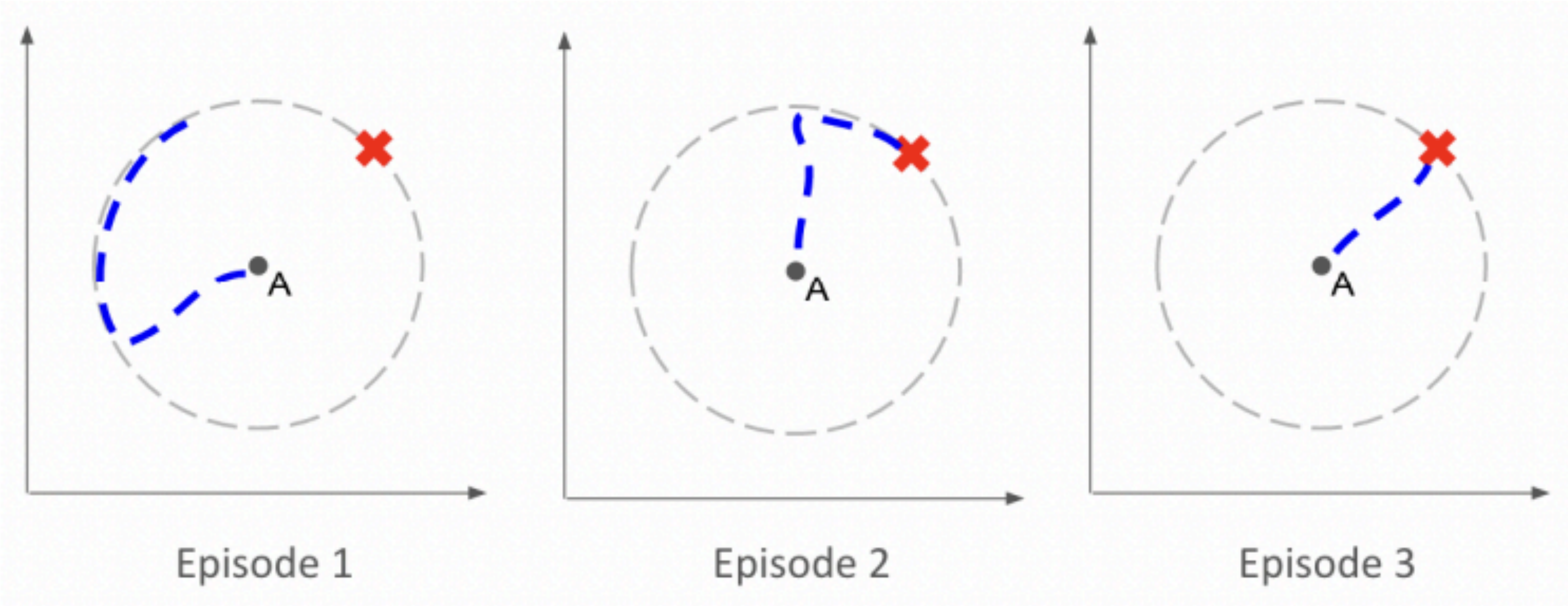
Proposed Solution

Component 4: Physics-Informed Deep Learning

- Meta-Reinforcement Learning (e.g. MAML) [3][5]: Speed & Generalization

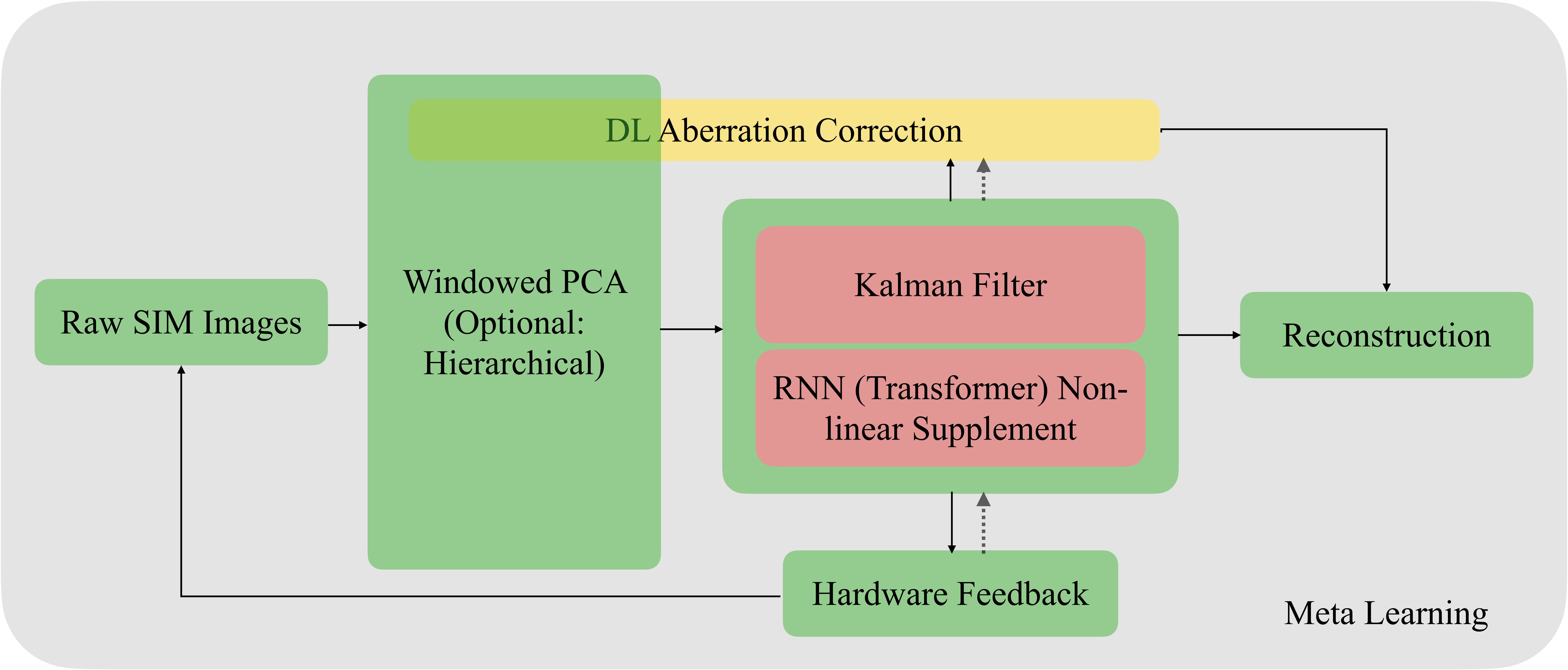


(a) Meta-Training Tasks



(b) Rollout at Meta-Test Time

System Architecture



Expected Results

- **Stability**: Robust parameter tracking over extended periods without manual recalibration.
- **Artifact Reduction**: Real-time correction eliminates common SIM artifacts like striping and moiré patterns that arise from parameter misestimation.
- **Extended Capabilities**: The physics-informed deep learning component should enable SIM in more challenging optical environments.

=> It maintains PCA-SIM's real-time performance while adding temporal robustness.

Challenges

Several technical challenges must be addressed:

- **Real-time Processing**: Attaining 10 fps for 512^2 images (11.1 fps for PCA-SIM) while incorporating all components requires careful optimization and GPU parallel acceleration.
- **Hardware Latency**: The feedback loop must minimize delays between parameter estimation and hardware adjustment to prevent system oscillations.
- **Validation Complexity**: Distinguishing between illumination drift and actual biological dynamics requires sophisticated protocols. Suitable validation metrics are of utmost importance.
- **Overfitting:** For training the deep learning component, we need sufficient diversity of aberration patterns to ensure generalization without overfitting.

References

[1] Rapid, artifact-reduced, image reconstruction for super-resolution structured illumination microscopy

https://www.the-innovation.org/article/doi/10.1016/j.xinn.2023.100425

[2] Parameter estimation of the structured illumination pattern based on principal component analysis (PCA): PCA-SIM

https://www.nature.com/articles/s41377-022-01043-9#:~:text=frequency%20vectors%20of%20the%20illumination,based%20parameter%20estimation

[3] Meta-rLLSM-VSIM: Meta Learning-Empowered Reflective Lattice Light-Sheet Virtual Structured Illumination Microscopy

https://github.com/Intelligent-SR-Imaging/Meta-rLLS-VSIM?tab=readme-ov-file

[4] STAR-RL: Spatial-temporal Hierarchical Reinforcement Learning for Interpretable Pathology Image Super-Resolution

https://arxiv.org/pdf/2406.18310v1

[5] A Survey of Meta-Reinforcement Learning

https://arxiv.org/pdf/2301.08028